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### Cell attachment polymer conjugate

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The present invention relates to a cell attachment polymer conjugate comprising an ultra-low attachment (ULA) polymer a) and one or more polypeptide(s) b) and to a device for cultivating cells, wherein the device comprises a surface, which is coated with such a cell attachment polymer.

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Most cells in higher organisms are embedded in a mesh of fibrous proteins and carbohydrates including a wealth of soluble signal molecules – called the extracellular matrix (ECM). The name “Collagen”, one of the major components of the complex extracellular network, is translatable from the French word “colle” for “glue” depicting one of the functions of the ECM: It is to ensure the correct location of a cell in several tissues.

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In the last years, it became clear that the ECM's role goes far beyond this function and is much more complex. Many cells cannot thrive unless surrounded by the ECM. The cells receive signals from the ECM by interaction of the cell receptors with proteins, peptides or soluble molecules activating cell signalling cascades which control the cells' behaviour. Thus, it becomes crucial for culturing cells in *in vitro* systems to mimic the natural environment of cells in the human body to ensure a similar cell behaviour.

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Plastic surfaces used in cell culturing are normally treated with plasma or a corona discharge to increase the hydrophilicity of the plastic surface. This modulates the tendency of the cells' surface proteins to adhere to the plastic surface. More advanced cell culture materials employ polymers to coat the plastic surface and reduce non-specific binding

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(NSB). Especially coating with polymeric surfactants, e.g. hydrophobic brush polymers or other hydrophobic anchoring groups linked to hydrophilic, often hydrophilic polymeric moieties, like derivatives of polyethylene glycol or polyacrylamides can reduce NSB to a minimum. The polymers when dissolved in water can coat the plastic surface of the vessels within a few seconds and create a highly polar surface so that even adherent cells can be cultured in suspension and form spheroids in plastic vessels, e.g. within the cavities of a microtiter plate. This indicates that cells have basically no affinity to such coated vessels. Such coatings are generally quite stable and can even be irradiated with hard beta-, gamma- or X-rays to create a sterile environment. Further, such surfaces have no tendency to interact with receptors on a cell in a way similar to the components of the ECM. Accordingly, low-NSB equipped surfaces are by no means suitable to mimic the influence of the ECM on cultured cells.

For this purpose, it is often practiced to implement the ECM in *in vitro* culture systems by isolating the regulating components of the ECM, absorb it to the surface of cell culture plasticware (polystyrene, polyolefin, polyacrylate etc.) and thus provide the stimulation signals to the cells to be cultures in an artificial vessel. Several commercially available products employ surfaces coated by collagen or other proteins of the ECM to the cultured cells to mimic the presence of the ECM.

Such products using whole isolated proteins or fragments of proteins from the ECM prepared by enzymatic cleavage come with distinct disadvantages. First, such products are often not xeno-free – that means the isolated proteins have animal- or human origins. This renders these products incompatible with most clinical applications. Also, lot-to-lot differences intrinsic to preparations of isolated natural proteins may constitute a challenge to using these products even for applications outside the clinical field. Secondly, coated proteins do not only provide a specific receptor-based interaction with the cells in culture but also cause non-specific interaction between the protein-coating of the vessel and the cells to be cultured. Such interaction can be based on lipophilic interaction between hydrophobic moieties on the surface of the cells and on the coated proteins or on other non-covalent and non-specific interactions. This can cause a non-desired stimulation of the cells causing undesired cellular effects, for example stronger or weaker proliferation.

Furthermore, the coating process of such proteins to labware like flasks or microtiter plates is difficult to implement: small differences in the purity of the peptides or proteins, pH variations of the coating buffers used, temperature differences while coating and other process variations can have a distinct impact on the stability, charge and conformation of

the proteins. This can impair the coating process itself or the performance of the derived products leading to lot-lot differences. Such cell culture products coated with specific proteins are usable to activate specific cell signalling pathways and control the cells' fate. Therefore, it is indispensable to avoid unspecific binding of cells to undesirable by-products.

5 Such by-products can be incorrectly folded proteins created in the isolation- or coating process.

Thus, there is a need for mimicking the natural environment of cells in the human body, preferably without the disadvantages described above.

10 The primary object of the present invention was thus to provide an improved possibility for culturing human cells. Particularly, an object of the present invention is to provide a coating material for cell culturing devices, which may advantageously mimic the natural environment of human cells.

15 The primary object of the present invention is solved by a polymer conjugate comprising or consisting of

a) an ultra-low attachment (ULA) polymer that carries one or more functional groups(s),  
20 wherein the, one, two, three or more or all functional groups(s) is/are selected from the group consisting of primary amines, carboxyl groups, aldehyde groups, alkyne, epoxide groups, hydroxyl groups, ketones, esters, amides, nitriles, thiols, hydroxylamines, imines, alkenes, isocyanates, halides, ether and azides. Additionally, the functional groups of the polymer can be further chemically modified  
25 to enable the conjugation of the one or more polypeptide(s),

and

b) one or more polypeptide(s), wherein the amount of amino acids in the polypeptide(s)  
30 is in a range of from 5 to 100 amino acids, preferably wherein the, one, two, three or more or all polypeptide(s) is/are selected from the group consisting of growth peptides, peptides stimulating cell differentiation, preferably stem cell differentiation, peptides preventing cell differentiation, preferably stem cell differentiation, peptides promoting cell adhesion, peptides promoting cell  
35 survival, peptides promoting cell proliferation, peptides promoting cell expansion, peptides supporting migration and/or motility, and peptides promoting cell cycle progression,

wherein the, one, two, three or more or all polypeptide(s) b) is/are conjugated to the ULA polymer a), wherein the conjugation is realized through the one or more functional groups(s) of polymer a).

- 5 The term "ultra-low attachment (ULA) polymer" as used herein is known to a skilled person and is frequently used in the state of the art.

Preferably, the term "ultra-low attachment (ULA) polymer" as used herein refers to a polymer, preferably a polymeric surfactant comprised of a hydrophobic brush polymer  
10 and/or other hydrophobic anchoring groups, linked to one or several or many hydrophilic moieties, like derivatives of polyethylene glycol or polyacrylamides which provides ultra-low attachment of cells when coated to a cell culture device.

Preferably, the ultra-low attachment is defined by that adhesive cells, preferably fibroblasts, particularly preferably mouse fibroblasts, preferably mouse fibroblasts of the 3T3-line, do  
15 not adhere to the polymer coated to a cell culture device.

Preferably the term "do not adhere" as used herein defines that at most 20 %, preferably at most 19 %, preferably at most 18 %, preferably at most 17 %, preferably at most 16 %, preferably at most 15 %, preferably at most 14 %, preferably at most 13 %, preferably at  
20 most 12 %, preferably at most 11 %, preferably at most 10 %, preferably at most 9 %, preferably at most 8 %, preferably at most 7 %, preferably at most 6 %, preferably at most 5 %, preferably at most 4 %, preferably at most 3 %, preferably at most 2 %, preferably at most 1 % of the number of cells, which was added to the cell culture device, adhere to the polymer, preferably the polymer coated surface.

25 Preferably the term "do not adhere" as used herein defines that at least 80 %, preferably at least 81 %, preferably at least 82 %, preferably at least 83 %, preferably at least 84 %, preferably at least 85 %, preferably at least 86 %, preferably at least 87 %, preferably at least 88 %, preferably at least 89 %, preferably at least 90 %, preferably at least 91 %, preferably at least 92 %, preferably at least 93 %, preferably at least 94 %, preferably at  
30 least 95 %, preferably at least 96 %, preferably at least 97 %, preferably at least 98 %, preferably at least 99 % of the number of cells, which was added to the cell culture device, do not adhere to the polymer, preferably the polymer coated surface.

35 Whether cells adhere to the ULA polymer or not can be determined by the morphology of the cells. For example, non-adhering fibroblasts have a round shaped morphology, whereas in adhering fibroblasts several cellular protrusions, increased cell area and an

elongated shape can be observed. Frequently, non-adhering cells may form spheroids, which can also be observed by morphological characterization of the cells.

Whether cells adhere to the ULA polymer, preferably the ULA polymer coated surface, or not is preferably determined within 24 hours after adding the cell to the cell culture device. Whether cells adhere to the ULA polymer or not is preferably determined 6, further preferably 12, particularly preferably 15, especially preferably 18, even further preferably 21, more preferably 24, most preferably 30 hours after adding the cell to the cell culture device.

Advantageously, culturing cells with a cell culture device coated with the conjugate according to the invention provides that the cells do not adhere through non-specific binding as they often do on untreated plastic surfaces. Still, they can interact through a specific binding of a cellular receptor with a peptide conjugated to the ULA polymer, preferably the ULA polymer coated surface. This is a clear difference to similar embodiments of the principle of offering peptides, protein fragments or whole proteins on a plastic surface. If the immobilization of the specific binding molecule has been realized on a ULA surface, e.g., a plastic surface coated with an ULA polymer – the interaction of the cells with the surface – is limited to a specific interaction, i.e. the interaction between the cellular receptor and the peptide. Any unspecific interaction is prevented or at least reduced by the ULA coating. Thus, the specific signalling effect can be studied without being overlaid by an unspecific interaction. Thus, with the conjugate according to the invention it is possible to mimic the natural environment of cells.

Preferably, the term “polymer” as used for describing the conjugate according to the invention, its features and effects, refers to a ULA polymer as described herein.

Further advantageously, as the employed conjugation procedures generally yield stable covalent bonds, also the coatings conjugated to peptides can be sterilized with beta- and gamma-radiation or x-rays.

Preferably, the one, two, three or more or all polypeptides exhibit a sequence homology of at least 70 %, preferably at least 80 %, preferably at least 90 %, preferably at least 91 %, preferably at least 92 %, preferably at least 93 %, preferably at least 94 %, preferably at least 95 %, preferably at least 96 %, preferably at least 97 %, preferably at least 98 %, preferably at least 99 % to a sequence of equal length of an integrin binding protein, preferably of collagen, laminin, tenascin, vitronectin or fibronectin.

It was found that the cell repellent properties of a low-NSB surface can be combined with the specific signalling properties characterizing the ECM in order to generate an environment that prevents unspecific interaction and allows for specific interaction through the conjugated polypeptides perfectly mimicking the ECM.

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Such desired effect – specific interaction through one peptide or two or more different peptides conjugated to the polymer combined with the non-interacting, cell repellent properties of the ULA surface - has distinct advantages: Adherence of the cultured cells to the coated surface through one or several specific receptors, stimulation of the specific  
10 receptors to confer the desired signalling to the cultured cells while avoiding or reducing unspecific interaction between the cells and the surface of the culture vessel. The latter effect prevents undesired adherence of the cells to the surface and undesired effects on the cells caused by such unspecific adherence.

15 The fact that several different peptides can be conjugated on the carrier ULA polymer or to a mixture of ULA polymers provides the advantage that the specific interaction between the coated surface and the cells can be spread out over two or more different receptors instead of interaction with just one receptor. In case cells change their properties while specifically bound to the vessel surface and a receptor gets lost or changed during culture,  
20 the cells can stay adhered through a second or third receptor. Such effect is particularly important when differentiating stem cells while bound to the coated surface: Stem cell differentiation is often linked to profound changes of cell properties, including receptor expression. This is why differentiating adhered stem cells are at high risk of the adhered cells separating of the coated surface during the differentiation process. This may cause  
25 loss of the cell during the following purification processes. Having the possibility to adhere the cells through more than one receptor increases the chances not to lose such cells.

Combining a ULA polymer with the stimulating peptides as described has another advantage: Provided the cell culture employing such combination is used to test the  
30 properties of small molecules – for example during experiments to develop new therapeutic drugs - such ULA polymer can reduce the ability of the small molecules to bind to the vessel surface – such as a plastic surface or a surface coated with proteins exposing a vast range of different polarities and differently charged moieties to the solution. An ULA surface with the ULA properties conferred by highly polar polyethylene glycol or polyacrylamide moieties  
35 can also prevent or reduce the absorption of small molecules to a surface, keep up the desired concentration of such small molecules by avoiding substance losses to the surface

and thus provide more realistic results on the effects a desired concentration of such small molecules will have on the cells.

It is thus preferred that the amount of amino acids in the polypeptide(s) b) of the cell attachment polymer conjugate according to the invention is in a range of from 5 to 95 amino acids, preferably in the range of from 5 to 90 amino acids, preferably in the range of from 5 to 85 amino acids, preferably in the range of from 5 to 80 amino acids, preferably in the range of from 5 to 75 amino acids, preferably in the range of from 5 to 70 amino acids, preferably in the range of from 6 to 65 amino acids, preferably in the range of from 6 to 60 amino acids, preferably in the range of from 6 to 55 amino acids, preferably in the range of from 6 to 50 amino acids, preferably in the range of from 6 to 45 amino acids, preferably in the range of from 6 to 40 amino acids, preferably in the range of from 7 to 35 amino acids, preferably in the range of from 7 to 30 amino acids.

However, it was also found that unlike proteins or fragments of proteins, smaller sized oligo- or polypeptides often cannot be coated directly to a plastic surface. The reason is that often there are not larger-size hydrophobic moieties that can be directly absorbed on non-polar plastic surfaces.

The polymer conjugate according to the invention, solves this problem: the polypeptide(s) is/are conjugated to the polymer, as described herein and thus anchors these polypeptides through the binding of the polymer to the surface and reduces the non-specific adherence of the cells to the vessel's surface. Furthermore, the interaction of the peptides with the surface receptors of the cells is possible. This means that this implementation allows to mimic the ECM.

However, at the same time, it is particularly advantageous to prevent or reduce non-specific binding as described herein. It is thus preferred that the ULA polymer a) is selected from the group consisting of block-copolymers, brush polymers (polyethylene glycol-polypropylene oxide or ethylene glycol-propylene oxide, n-Butylmethacrylat with methacrylic acid or alkyl-containing brush polymers (copolymers of Poly-(ethylenglykol)-methylether-methacrylat and propargyl acrylate)), alternating styrene maleic anhydride copolymer (esterified block- copolymer).

Chemical compounds particularly useful to serve as Ultra-low attachment (ULA) polymers typically have the following structural components: One, several or many moieties with low polarity: aliphatic chains, siloxanes, aromatic units on a polymeric chain, often forming the

backbone of the polymer or bound to the backbone of the polymer. The second component are highly polar moieties like polyethylene glycol or polyacrylamides connected to the ends of the hydrophobic backbone or grafted alongside of it. Also, the polymer needs to carry chemical groups that allow to conjugate the peptides to the polymer. If such reactive groups  
5 confer a charge to the polymer, be it permanent or pH-dependant, like carboxy- or amino-groups, it can be necessary to protect such groups with an excess of a charge neutralizing agent to preserve the ULA properties of the polymer. Using reactive groups, for example hydroxy-groups, to conjugate the peptides that do not confer charge to the polymer may be better suited to preserve its polarity.

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Useful ULA polymers are siloxane based anchor polymers with polyethylene glycol or polyethylene glycol and isopropylene glycol side chains, like Tegoprens (from Evonik, Essen, Germany), Silwet L7200, Silwet L7230, Silwet L7657 (all from Momentive, Albany, NY, USA), oligomeric surfactants like Brij35 (Merck, Darmstadt, Germany), or graft  
15 polymers like Inutec SL1 (Nordmann, Rassmann GmbH, Hamburg, Germany) or copolymers prepared from n-Butylmethacrylate (nBMA), Methacrylic acid and Methoxy Polyethyleneglycol Methacrylate (Bisomer S20W), hereinafter named faCellitate F210.

All such compounds are amphiphilic, readily available compounds absorbing to non-polar  
20 surfaces and ULA-properties conferred by highly polar side chains or highly polar scaffold moieties with the polymer carrying functional groups ready to be conjugated with biological receptors like peptides, proteins or nucleic acids.

As described herein, the, one, two, three or more or all functional groups(s) of the polymer  
25 is/are selected from the group consisting of primary amines, carboxyl groups, aldehyde groups, alkynes, epoxide groups, hydroxyl groups, ketones, esters, amides, nitriles, thiols, hydroxylamines, imines, alkenes, isocyanates, halides, ether and azides.

The term "a polymer carrying one or more functional groups" is thus to be understood such  
30 that the functional groups(s) is/are present in at least one of the monomer(s) of the polymer or at the end of the polymer or at branches of the polymer, wherein the functional groups of at least one monomer in the polymer or at the end of the polymer or at branches of the polymer is/are not binding to another monomer. Thus, in the polymer, these functional group(s) or at least one, at least two, at least three or all of these functional groups(s) are  
35 available for binding to another compound, such as one or more polypeptide(s) b).

The term “wherein the conjugation is located at the one or more functional groups(s) of polymer a)” describes the conjugation of the one or more polypeptide(s) with the polymer. This term is to be understood such that the conjugation of the, one, two, three or more or all of the polypeptide(s) with the polymer is obtained by binding of the, one, two, three or more or all of the polypeptide(s) to such a functional group of the polymer. Thus, the, one, two, three or more or all of said functional groups(s) of the polymer are bound to the one or more polypeptide(s).

Preferably, the conjugation results in covalent bonds.

Thus, for providing such a conjugation, the polymer needs to carry such functional groups(s). The, one, two, three or more or all functional groups(s) is/are selected from the group consisting of primary amines, carboxyl groups, aldehyde groups, alkynes, epoxide groups, hydroxyl groups, ketones, esters, amides, nitriles, thiols, hydroxylamines, imines, alkenes, isocyanates, halides, ether and azides.

For example, the primary amines can be reacted with a heterobifunctional linker like SMCC or sulfo-SMCC (SMCC: (Sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexan-1-carboxylat); Thermo Fisher Scientific Product No. 22322). This procedure can be carried out in aqueous solutions and converts the primary amine into an amid linked to a maleimide functionality. This maleimide then reacts in a Hetero-Michael-Addition with the thiol group in the cysteine moiety of a polypeptide tailored to bind to a receptor on the cells' surface.

Similarly, carboxy groups can be activated with derivatives of carbodiimide, e.g. EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) to form a reactive urea intermediate that can directly react with a free amine in lysine moieties of a peptide to form a stable amide bond.

Aldehyde groups in a coating polymer can react with amines in the peptides to form Schiff bases that can then be stabilized by a subsequent reduction.

Epoxides can be reacted with alcohols or primary or secondary amines for form ethers or secondary or tertiary amines – all perfectly suited to form stable conjugation bonds.

Alkynes can be reacted with azidegroup bearing compounds to yield a stable triazole-linkage.

These reactions can be carried out within less than one hour at a moderate pH and yield stable products. The conjugate comprising or consisting of the peptides and the coating polymer can be purified by ultra-filtration, size-exclusion chromatography, dialysis or other procedures based on the different size or charge of the different components in the reaction. The resulting polymer conjugate can be absorbed to a plastic surface. Advantageously, the polymer conjugate can also be attached to non-plastic surfaces. In particular, anchoring to metal surfaces can be achieved through thiol-containing moieties or phosphate containing moieties or phosphonic acid containing moieties. The binding on glass surfaces, normally characterized by partially deprotonated Si-O-H binding can be achieved by positively charged moieties like partially protonated amino-groups or boronic acid groups.

In this way, the conjugation process may be further controlled and may provide advantageously constant results.

Preferably, the, one, two, three or more or all polypeptide(s) b) is/are or correspond to fragments of proteins of the human extracellular matrix (ECM), preferably wherein the, one, two, three or more or all proteins of the human extracellular matrix is/are selected from the group consisting from integrin binding proteins, such as Collagen, Laminin, Vitronectin, Tenascin, Fibronectin, Collagen I, Fibronectin- RGD (linear and cyclic), PRARI (SEQ ID NO.: 1), PHSRNGGRGD (SEQ ID NO.: 2), Laminin (SEQ ID NO.:3), KAFDITYVRLKF (SEQ ID NO.: 4), Vitronectin (SEQ ID NO.: 5), and Bone-Sialoprotein (SEQ ID NO.: 6). Preferably, other protein or peptide sequences are also be of interest, for example RKKGRKKGRKKGRKK (SEQ ID NO. 7) or a peptide according to any one of SEQ ID Nos.: 8 to 18.

The term "correspond to fragments of proteins" is to be understood such that these polypeptide(s) do not need to be obtained from such a protein. However, it is preferred that the polypeptide(s) comprise or consist of an amino acid sequence being identical or having at least 60%, preferably at least 65%, preferably at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at least 93%, preferably at least 94%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, preferably at least 99%, sequence identity to the amino acid sequence of a fragment of such a protein.

Preferably, whenever the present disclosure refers to sequence identities or homologies of amino acid sequences in terms of percentages, such references are to values as can be calculated using EMBOSS Water Pairwise Sequence Alignments (proteins) ([http://www.ebi.ac.uk/Tools/psa/emboss\\_water/](http://www.ebi.ac.uk/Tools/psa/emboss_water/)) for amino acid sequences, respectively.

5 Local sequence alignment tools provided by the European Molecular Biology Laboratory (EMBL) European Bioinformatics Institute (EBI) use a modified Smith-Waterman algorithm (see <http://www.ebi.ac.uk/Tools/psa/> and Smith, T.F. & Waterman, M.S. "Identification of common molecular subsequences" Journal of Molecular Biology, 1981 147 (1):195-197). Furthermore, here, when performing the respective pairwise alignment of two sequences

10 using the modified Smith-Waterman algorithm, reference is made to the default parameters currently given by EMBL-EBI. These are for amino acid sequences: Matrix = BLOSUM62, Gap open penalty = 10 and Gap extend penalty = 0.5.

Preferably, the term fragment of a protein refers to a section of the amino acid sequence

15 of the protein.

It was surprisingly found that the obtained cell attachment polymer conjugate can be coated to a device for cultivating cells to provide a low-NSB surface with signalling properties towards adjacent cells. These signalling properties are well reproducible as they are

20 conferred by well characterized synthetic peptides and they are not affected by lot-to-lot differences of proteins isolated from natural sources.

Thus, with a cell attachment polymer according to the invention, the presence of the ECM can be advantageously mimicked.

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The present invention further relates to a cell attachment polymer conjugate, preferably as described herein, obtained or obtainable by a method comprising the steps:

- 30 i) providing a ULA polymer carrying one or more functional groups(s), wherein the, one, two, three or more or all functional groups(s) is/are selected from the group consisting of primary amines, carboxyl groups, aldehyde groups, epoxide groups, hydroxyl groups, ketones, esters, amides, nitriles, thiols, hydroxylamines, imines, alkenes, isocyanates, halides, ether and azides,
- 35 ii) providing one or more polypeptide(s), wherein the amount of amino acids in the polypeptide(s) is in a range of from 5 to 100 amino acids,

5 wherein the, one, two, three or more or all polypeptide(s) is/are selected from the group consisting of growth peptides, peptides stimulating cell differentiation, preferably stem cell differentiation, peptides preventing cell differentiation, preferably stem cell differentiation, peptides promoting cell adhesion, peptides promoting cell survival, peptides promoting cell proliferation, peptides promoting cell expansion, peptides supporting migration and/or motility, and peptides promoting cell cycle progression,

10 iii) conjugating the, one, two, three or more or all of the polypeptide(s) provided in step ii) to the or a polymer provided in step i).

Preferably, providing a polymer in step i) may comprise or consist of

15 ia) providing one or more monomer(s), preferably carrying one or more functional groups(s), wherein the, one, two, three or more or all functional groups(s) is/are selected from the group consisting of primary amines, carboxyl groups, aldehyde groups, epoxide groups, hydroxyl groups, ketones, esters, amides, nitriles, thiols, hydroxylamines, imines, alkenes, isocyanates, halides, ether and azides,

20 ib) polymerizing the, one, two, three or more or all of the monomer(s) provided in step ia) to obtain a polymer.

25 The present invention further relates to a device for cultivating cells wherein the device comprises a surface, which is coated with a cell attachment polymer according to the invention.

Devices for cultivating cells are known to a skilled person. Such a device may be any device that is suitable for cultivating cells and/or for handling such cell cultures.

30 Such a device may be selected from the group consisting of include flasks (e.g. round bottom flasks, spinner flasks, Erlenmeyer flasks, retorts, Florence flasks), cell culture dishes, cell culture bottles, cell culture bags, pipette tips, petri dishes, multiwell plates, reactors, especially bioreactors, tubes, pipettes, syringes, chips, slides, and cell culture scaffolds (such as microcarriers).

35 Preferably, the surface of the device is made of a material, which comprises or consists of a material selected from the group consisting of glass, quartz, silicon, metals, metal oxides,

and organic polymers (e.g. polycarbonate, polystyrene, hydrophilized polystyrene, polyamide, poly(methyl methacrylate), polyesters, polyvinylchloride, polyvinylidene chloride, polymers comprising fluorinated monomers like fluorinated ethylene or propylene, polyolefines (such as polyethylene (like low density polyethylene, ultralow density  
5 polyethylene, linear low density polyethylene, high density polyethylene, high molecular weight polyethylene, ultrahigh molecular weight polyethylene), polypropylene, (like oriented polypropylene, biaxially oriented polypropylene), polynorbornene, cyclic olefin polymers (COP) or cyclic olefin copolymers (COC) such as copolymers of ethylene and norbornene)).

10 Preferably, the surface of the device is made of a material, which comprises or consists of a material selected from the group consisting of polyolefines, in particular polystyrene, polyethylene, polypropylene, and (partially) fluorinated polyolefines such as fluorinated ethylene propylene.

15 Preferably, the surface of the device is made of a material, which comprises or consists of polystyrene.

The present invention further relates to a method for producing a device according to the invention comprising the steps

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- a) providing a device for cultivating cells,
- b) providing a polymer conjugate according to the invention, and
- 25 c) coating the device, preferably the surface of the device, provided in step a) with the polymer conjugate provided in step b).

Preferably, the surface of the device refers to the or a part of the device, which will be in contact (via the coating) with cells when using the device for cultivating cells.

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Preferably, the term "coating the device" refers to the typical procedure for coating a device to be used for cultivating cells. Such a procedure is well known to a skilled person. Typically, for coating a device, the coating material is added on the surface to be coated and after a period of time, preferably in the range of from 1 min to 24 h, preferably in the range of from  
35 5 min to 12 h, preferably in the range of from 10 min to 4 h, preferably in the range of from 15 min to 2 h, the surface is washed to remove the non-coated coating material.

The present invention further relates to a method for cultivating cells, comprising the steps

- a) providing a device according to the invention,
- 5 b) adding cells to the device provided in step a) and
- c) cultivating the cells added in step b) in the device.

Furthermore, the present invention relates to the use of a device according to the invention  
10 for cultivating cells.

The cells to be cultivated, as described herein, may be selected from adhesive cells and/or non-adhesive cells. The term "adhesive cells and non-adhesive cells" refers to a mixture of adhesive cells and non-adhesive cells. Preferably, the cells are selected from eukaryotic  
15 cells, somatic cells, primary cells, cells grown from immortalized cell lines, induced pluripotent stem cells, hematopoietic stem cells and mesenchymal stem cells.

As described above, it was surprisingly found that the device according to the invention provides a low-NSB surface with signalling properties towards adjacent cells. These  
20 signalling properties are well reproducible as they are conferred by well characterized synthetic peptides and they are not affected by lot-to-lot differences of proteins isolated from natural sources.

Thus, with a device according to the invention, the presence of the ECM can be  
25 advantageously mimicked.

The invention is further characterized in the following with exemplary, non-limiting examples.

### Examples

Reagents and chemicals were purchased from abcr, Carl Roth, ChemPur, Carbolution and Merck: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (Carl Roth, order number 2156.2),  
5 *N*-hydroxysulfo-succinimide (Carbolution, order number CC01035), 1-(2-aminoethyl)maleimide hydrochloride (Chempur, order number BD76191-1g), hydrazine monohydrate (Merck, order number 207942-5G), Silwet L7657 (Momentive, Albany, NY), Dbco-amine tfa (abcr, order number AB488945), 2-[2-(2-propynyloxy)ethoxy]ethylamine (abcr, order number AB474698). The peptides were synthesized by NovoPro Bioscience  
10 Inc and ProteoGenix. Dde refers to (*N*-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl)). All reagents and chemicals were used as purchased unless stated otherwise.

### Manufacturing of a cell attachment polymer conjugate of the invention

#### 15 **Example 1: Manufacturing of ULA faCellitate F210**

As initial batch, ethanol (410.97g), Bisomer S20W (50% in water, 32.09g), *n*-butylmethacrylate (3.29g) and methacrylic acid (0.66g) are added in a 2L reactor with an anchor stirrer. During the whole reaction, the reactor is purged with nitrogen (10 L/min) and  
20 stirred at 125 rpm. At the beginning of the reaction, the reactor is heated to 80°C in 30 min. When reaching 80°C, *tert*-butylperoxypivate (75% in isododecane, 0.27g) in ethanol (9.73g) is added after 1 min. After 5 min stirring, a mixture of ethanol (125.59g), Bisomer S20W (50% in water, 288.83g), *n*-butylmethacrylate (29.61g) and methacrylic acid (5.98g) was added continuously in 3h. Simultaneously *tert*-butylperoxypivate (75% in  
25 isododecane, 2.40g) in ethanol (87.60g) is added over a time period of 3,5 h. Subsequently, the reaction is polymerized for another 2h. Then ethanol (20.33g) and *tert*-butylperoxypivate (75% in isododecane, 2.67g) are added continuously over 1 h. At the end, polymerization was continued for another 2 hours and then the reaction mixture was cooled down as quickly as possible to 25°C. The next day the reactor is heated to 130°C  
30 (bath temperature). Simultaneously, water steam distillation was conducted until the unreacted monomers had been removed. The distillation ends at an internal temperature of 100°C. Then the distillation is stopped and the reaction is cooled down to 25°C as quickly as possible.

Solvents were removed with a rotary evaporator under reduced pressure. The resulting  
35 watery slurry was freeze dried until a colorless to yellowish solid was obtained.

Polymer-peptide conjugates to coat surfaces for induced pluripotent stem cell (iPSC) cultivation

**Example 2: Preparation of polymer-peptide conjugate via thiol-maleimide Michael addition**

faCellitate F210 (500 mg, 643  $\mu$ mol, 1.00 equiv.) was dissolved in PBS buffer (20.0 mL) at 21 °C. Subsequently, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.95 g, 10.2 mmol, 15.9 equiv.) and *N*-hydroxysulfosuccinimide (1.11 g, 5.10 mmol, 7.93 equiv.) were added at 21 °C. The reaction mixture was cooled down to 0 °C and stirred for 15 min before adding 1-(2-aminoethyl)maleimide hydrochloride (450 mg, 2.55 mmol, 3.97 equiv.). The reaction mixture was stirred overnight and allowed to warm up to 21 °C. After submitting the mixture to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH4) and freeze-drying a colourless solid was obtained.

Part of this product (200 mg, 222  $\mu$ mol, 1.00 equiv.) was dissolved in PBS buffer (10.0 mL) at 21 °C before adding the peptides c(RGDyC) (52.4 mg, 88.1  $\mu$ mol, 0.397 equiv.), Ac-GCWGGPQVTRGDVFTMP-CONH<sub>2</sub> (81.5 mg, 44.1  $\mu$ mol, 0.199 equiv., SEQ ID NO.: 8) and Ac-GCWGRKKGRKKGRKKGRKK-CONH<sub>2</sub> (20.1 mg, 8.80  $\mu$ mol, 0.0396 equiv., SEQ ID NO.: 9) dissolved in dimethylsulfoxide (3.0 mL). The reaction mixture was stirred over night at 21 °C and submitted to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7). After freeze-drying a colourless solid was obtained.

**Example 3: Preparation of polymer-peptide conjugate via direct peptide coupling to the polymer**

faCellitate F210 (50.0 mg, 64.3  $\mu$ mol, 1.00 equiv.) was dissolved in PBS buffer (5.0 mL) at 21 °C. Subsequently, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (195 mg, 1.02 mmol, 15.9 equiv.) and *N*-hydroxysulfosuccinimide (111 mg, 510  $\mu$ mol, 7.93 equiv.) were added at 21 °C. The reaction mixture was cooled down to 0 °C and stirred for 15 min before adding the peptides c(RGDyK) (30.0 mg, 48.4  $\mu$ mol, 0.753 equiv.), Ac-GKWGGPQVTRGDVFTMP-CONH<sub>2</sub> (45.0 mg, 23.9  $\mu$ mol, 0.372 equiv., SEQ ID NO.: 8) and Ac-GKWGRK(Dde)K(Dde)GRK(Dde)K(Dde)GRK(Dde)K(Dde)GRK(Dde)K(Dde)-CONH<sub>2</sub> (17.0 mg, 4.69  $\mu$ mol, 0.0729 equiv., SEQ ID NO.: 10) dissolved in dimethylsulfoxide/distilled water (1:1, 2.0 mL). The reaction mixture was stirred overnight

and allowed to warm up to 21 °C. After submitting the mixture to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-drying a colourless solid was obtained.

- 5 The product was then subjected to Dde deprotection by applying a hydrazine monohydrate solution in *N,N*-dimethylformamide (2% v/v) and stirring for 30 minutes at 21 °C. The reaction mixture was again purified by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-dried to obtain a colourless solid.

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**Example 4: Preparation of polymer-peptide conjugate via strain-promoted azide alkyne cycloaddition**

- faCellitate F210 (50.0 mg, 64.3 µmol, 1.00 equiv.) was dissolved in PBS buffer (5.0 mL) at 21 °C. Subsequently, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (246 mg, 1.29 mmol, 20.0 equiv.) and *N*-hydroxysulfosuccinimide (140 mg, 643 µmol, 10.0 equiv.) were added at 21 °C. The reaction mixture was cooled down to 0 °C and stirred for 15 min before adding Dbco-amine tfa (48.0 mg, 122 µmol, 1.90 equiv.) dissolved in dimethylsulfoxide (1.5 mL). The reaction mixture was stirred overnight and allowed to warm up to 21 °C. After submitting the mixture to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-drying a colourless solid was obtained. The product (30.0 mg, 28.9 µmol, 1.00 equiv.) was dissolved in PBS buffer (5.0 mL) at 21 °C before adding the peptides cyclo(RGDDYK(N<sub>3</sub>)) (11.7 mg, 18.1 µmol, 0.625 equiv., SEQ ID NO.: 12), Ac-GK(N<sub>3</sub>)-WGGPQVTRGDVFTMP-CONH<sub>2</sub> (17.2 mg, 9.03 µmol, 0.313 equiv., SEQ ID NO.: 11), Ac-GK(N<sub>3</sub>)-WGRKKGRKKGRKKGRKK-CONH<sub>2</sub> (4.23 mg, 1.81 µmol, 0.0625 equiv., SEQ ID NO.: 13) dissolved in dimethylsulfoxide (2.0 mL). The reaction mixture was stirred over night at 21 °C and submitted to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7). After freeze-drying a colourless solid was obtained.

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**Example 5: Preparation of polymer-peptide conjugate via thiol-yne reaction**

- faCellitate F210 (300 mg, 386 µmol, 1.00 equiv.) was dissolved in PBS buffer (10.0 mL) at 21 °C. Subsequently, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.48 g, 7.71 mmol, 20.0 equiv.) and *N*-hydroxysulfosuccinimide (837 mg, 3.86 mmol, 10.0 equiv.) were added at 21 °C. The reaction mixture was cooled down to 0 °C and stirred for 15 min before adding

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2-[2-(2-propynyloxy)ethoxy]ethylamine (248 mg, 1.74 mmol, 4.50 equiv.). The reaction mixture was stirred over night and allowed to warm up to 21 °C. After submitting the mixture to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-drying a colourless solid was obtained.

Under argon atmosphere, part of this so obtained product (50.0 mg, 55.3 µmol, 1.00 equiv.), 2,2'-azobis(isobutyronitrile) (1.0 mg, 5.53 µmol, 0.100 equiv.) and the peptides c(RGDyC) (20.6 mg, 34.6 µmol, 0.625 equiv.), Ac-GCWGGPQVTRGDVFTMP-CONH<sub>2</sub> (32.0 mg, 17.3 µmol, 0.313 equiv., SEQ ID NO.: 8) and Ac-GCWGRKKGRKKGRKKGRKK-CONH<sub>2</sub> (7.89 mg, 3.46 µmol, 0.0625 equiv., SEQ ID NO.: 9) were dissolved in anhydrous *N,N*-dimethylformamide (5.0 mL) and stirred for two days at 60 °C. Subsequently, the mixture was purified by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-dried to yield a colourless solid.

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#### **Example 6: Preparation of polymer-peptide conjugate via thiol-yne reaction**

Under argon atmosphere, Silwet L-7657 (1.00 equiv.) and sodium hydride (1.50 equiv.) were dissolved in anhydrous tetrahydrofuran at 21 °C. Subsequently, propargyl bromide (2.00 equiv.) was added dropwise over the course of 20 minutes. The reaction mixture was stirred at 21 °C for 24 hours before adding distilled water at 0 °C. Afterwards, tetrahydrofuran was removed under reduced pressure and the remaining mixture was purified by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-dried to yield a colourless solid.

Under argon atmosphere, part of this so obtained product (1.00 equiv.), 2,2'-azobis(isobutyronitrile) (0.100 equiv.) and the peptides c(RGDyC) (0.625 equiv.), Ac-GCWGGPQVTRGDVFTMP-CONH<sub>2</sub> (0.313 equiv., SEQ ID NO.: 8) and Ac-GCWGRKKGRKKGRKKGRKK-CONH<sub>2</sub> (0.0625 equiv., SEQ ID NO.: 9) were dissolved in anhydrous *N,N*-dimethylformamide (5.0 mL) and stirred for two days at 60 °C. Subsequently, the mixture was purified by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-dried to yield a colourless solid.

Polymer-peptide conjugates for culturing Mesenchymal Stem Cells (MSCs)

**Example 7: Preparation of polymer-peptide conjugate via thiol-maleimide Michael addition**

- 5 faCellitate F210 (500 mg, 643  $\mu$ mol, 1.00 equiv.) was dissolved in PBS buffer (20.0 mL) at 21 °C. Subsequently, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.95 g, 10.2 mmol, 15.9 equiv.) and N-hydroxysulfosuccinimide (1.11 g, 5.10 mmol, 7.93 equiv.) were added at 21 °C. The reaction mixture was cooled down to 0 °C and stirred for 15 min before adding
- 10 1-(2-aminoethyl)maleimide hydrochloride (450 mg, 2.55 mmol, 3.97 equiv.). The reaction mixture was stirred over night and allowed to warm up to 21 °C. After submitting the mixture to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH4) and freeze-drying a colourless solid was obtained.
- 15 Part of this so obtained product (200 mg, 222  $\mu$ mol, 1.00 equiv.) was dissolved in PBS buffer (10.0 mL) at 21 °C before adding the peptide Ac-GCWGGRGDSP-CONH<sub>2</sub> (160 mg, 155  $\mu$ mol, 0.700 equiv., SEQ ID NO.: 14) or Ac-GCWGGGFOGER-CONH<sub>2</sub> (183 mg, 155  $\mu$ mol, 0.700 equiv., SEQ ID NO.: 15). The reaction mixture was stirred over night at 21 °C and submitted to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5
- 20 membrane, 5.000 MWCO, washing solution: distilled water, pH7). After freeze-drying a colourless solid was obtained.

**Example 8: Preparation of polymer-peptide conjugate via direct peptide coupling to the polymer**

- 25 faCellitate F210 (50.0 mg, 64.3  $\mu$ mol, 1.00 equiv.) was dissolved in PBS buffer (5.0 mL) at 21 °C. Subsequently, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (195 mg, 1.02 mmol, 15.9 equiv.) and N-hydroxysulfosuccinimide (111 mg, 510  $\mu$ mol, 7.93 equiv.) were added at 21 °C. The reaction mixture was cooled down to 0 °C and stirred for 15 min before adding
- 30 the peptide Ac-GKWGGRGDSP-CONH<sub>2</sub> (81.6 mg, 77.2  $\mu$ mol, 1.20 equiv., SEQ ID NO.: 16) or Ac-GKWGGGFOGER-CONH<sub>2</sub> (93.0 mg, 77.2  $\mu$ mol, 1.20 equiv., SEQ ID NO.: 15). The reaction mixture was stirred overnight and allowed to warm up to 21 °C. After submitting the mixture to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-drying a
- 35 colourless solid was obtained.

**Example 9: Preparation of polymer-peptide conjugate via strain-promoted azide alkyne cycloaddition**

faCellitate F210 (50.0 mg, 64.3  $\mu$ mol, 1.00 equiv.) was dissolved in PBS buffer (5.0 mL) at 21 °C. Subsequently, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (246 mg, 1.29 mmol, 20.0 equiv.) and *N*-hydroxysulfosuccinimide (140 mg, 643  $\mu$ mol, 10.0 equiv.) were added at 21 °C. The reaction mixture was cooled down to 0 °C and stirred for 15 min before adding Dbco-amine tfa (48.0 mg, 122  $\mu$ mol, 1.90 equiv.) dissolved in dimethylsulfoxide (1.5 mL). The reaction mixture was stirred overnight and allowed to warm up to 21 °C. After submitting the mixture to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-drying a colourless solid was obtained. The product (30.0 mg, 28.9  $\mu$ mol, 1.00 equiv.) was dissolved in PBS buffer (5.0 mL) at 21 °C before adding the peptide Ac-G-K(N<sub>3</sub>)-WGGRGDSP-CONH<sub>2</sub> (31.3 mg, 28.9  $\mu$ mol, 1.00 equiv., SEQ ID NO.: 17) or Ac-G-K(N<sub>3</sub>)-WGGGFOGER-CONH<sub>2</sub> (36.0 mg, 28.9  $\mu$ mol, 1.00 equiv.), SEQ ID NO.: 18. The reaction mixture was stirred over night at 21 °C and submitted to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7). After freeze-drying a colourless solid was obtained.

**Example 10: Preparation of polymer-peptide conjugate via thiol-yne reaction**

faCellitate F210 (300 mg, 386  $\mu$ mol, 1.00 equiv.) was dissolved in PBS buffer (10.0 mL) at 21 °C. Subsequently, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.48 g, 7.71 mmol, 20.0 equiv.) and *N*-hydroxysulfosuccinimide (837 mg, 3.86 mmol, 10.0 equiv.) were added at 21 °C. The reaction mixture was cooled down to 0 °C and stirred for 15 min before adding 2-[2-(2-propynyloxy)ethoxy]ethylamine (248 mg, 1.74 mmol, 4.50 equiv.). The reaction mixture was stirred overnight and allowed to warm up to 21 °C. After submitting the mixture to purification by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-drying a colourless solid was obtained.

Under argon atmosphere, part of this so obtained product (50.0 mg, 55.3  $\mu$ mol, 1.00 equiv.), 2,2'-azobis(isobutyronitrile) (1.0 mg, 5.53  $\mu$ mol, 0.100 equiv.) and the peptide Ac-GCWGGRGDSP-CONH<sub>2</sub> (57.1 mg, 55.3  $\mu$ mol, 1.00 equiv., SEQ ID NO.: 14) or Ac-GCWGGGFOGER-CONH<sub>2</sub> (65.2 mg, 55.3  $\mu$ mol, 1.00 equiv., SEQ ID NO.: 15) were dissolved in *N,N*-dimethylformamide (5.0 mL) and stirred for two days at 60 °C. Subsequently, the mixture was purified by ultrafiltration (Amicon® stirred cell, Ultracel® PL-

5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-dried to yield a colourless solid.

**Example 11: Preparation of polymer-peptide conjugate *via* thiol-yne reaction**

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Under argon atmosphere, Silwet L-7657 (1.00 equiv.) and sodium hydride (1.50 equiv.) were dissolved in anhydrous tetrahydrofuran at 21 °C. Subsequently, propargyl bromide (2.00 equiv.) was added dropwise over the course of 20 minutes. The reaction mixture was stirred at 21 °C for 24 hours before adding distilled water at 0 °C. Afterwards, tetrahydrofuran was removed under reduced pressure and the remaining mixture was purified by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-dried to yield a colourless solid.

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Under argon atmosphere, part of this so obtained product (1.00 equiv.), 2,2'-azobis(isobutyronitrile) (0.100 equiv.) and the peptide Ac-GCWGGRGDSP-CONH<sub>2</sub> (1.00 equiv., SEQ ID NO.: 14) or Ac-GCWGGGFOGER-CONH<sub>2</sub> (1.00 equiv., SEQ ID NO.: 15) were dissolved in *N,N*-dimethylformamide (5.0 mL) and stirred for two days at 60 °C. Subsequently, the mixture was purified by ultrafiltration (Amicon® stirred cell, Ultracel® PL-5 membrane, 5.000 MWCO, washing solution: distilled water, pH7) and freeze-dried to yield a colourless solid.

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**Example 12: Coating of a cell culture article with a cell attachment polymer conjugate of the invention**

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1. Dissolve the lyophilized polymer-peptide conjugate from either one of examples 2 to 11 in DPBS buffer to reach a concentration of 16 mg/ml (10X stock solution)

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2. Prepare the working solution by diluting the stock solution 1:10 in DPBS buffer (working concentration: 1.6 mg/ml). Be aware that the working solution needs to be freshly prepared, just before starting surface coating.

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3. Add 1 ml of working solution to one well of a 6-well plate (#351146, Corning). Other cell culture consumables can be used, such as 24-well plates, 96-well plates, and T75 flasks. If using other formats, adapt the volume of coating solution according to the dimension of the well/flask (e.g., 500 µl in one well of

a 24-well plate). Be aware that the surface needs to be non-tissue-cultured treated polystyrene.

4. Incubate for 1 hour at room temperature.
5. Discard the working solution and discard it.
6. Wash the surface three times with 1 ml of DPBS buffer.
- 10 7. Store the coated plate at – 18 °C or lower; subsequent cultivation of cells as shown in example 13.

**Example 13: Culturing cells with the cell culture article according to the invention**

- 15 Prepare cell suspension by resuspending BIONI010-B cells (human induced pluripotent stem cell line) in 2 ml of mTeSR+ medium (Stemcell Technologies).
- 20 1. Add 250,000 cells in one well of a 6-well plate coated according to example 12; for a control experiment use a non-coated polystyrene cell culture plate. Incubate cells at 37°C with 5% CO<sub>2</sub>.
  2. After 24 hours, monitor cell attachment using a bright field microscope.
  - 25 3. Monitor cell confluency (as indirect measurements of cell proliferation) and cell morphology using a bright field microscope every day until the culture reaches a confluency of 60-80% (usually after 7 days).
  - 30 4. Detach cells using the CTST<sup>™</sup> Versene<sup>™</sup> solution (#15040066, Thermo Fisher) according to the manufacturer instructions.
  5. Determine the expression of pluripotency markers:
    - hNANOG und hTRA1-81 at protein level by flow cytometry
    - hOCT4, hSOX2 und hNANOG at transcription level by RT-PCR

Results: All seeded cells adhered specific to the peptides presented on the coated surface. Cells appeared round and growth in colonies with clearly defined margins. 7 days after seeding the culture reached 70% confluency and 2,000,000 cells were counted. 90-100% cells were positive for the analyzed pluripotency markers both at

5 protein and transcription level.

Control: BIONI010-B cells were seeded on a tissue-culture treated polystyrene surface without coating with the polymer-peptide conjugate. None of the seeded cells attached to the surface. Cells formed multiple spheroids that floated within the well.

10 Most spheroids were lost during media changes. The few remaining spheroids were dissociated after 7 days of culture to determine the expression of pluripotency markers. Less than 30% of cells were positive for the analyzed markers.

Claims

1. Cell attachment polymer conjugate of
  - a) an ultra-low attachment (ULA) polymer carrying one or more functional groups(s), wherein the, one, two, three or more or all functional groups(s) is/are selected from the group consisting of primary amines, carboxyl groups, aldehyde groups, alkynes, epoxide groups, hydroxyl groups, ketones, esters, amides, nitriles, thiols, hydroxylamines, imines, alkenes, isocyanates, halides, ether and azides,

and

  - b) one or more polypeptide(s), wherein the amount of amino acids in the polypeptide(s) is in a range of from 5 to 100 amino acids,

wherein the, one, two, three or more or all polypeptide(s) is/are selected from the group consisting of growth peptides, peptides stimulating cell differentiation, preferably stem cell differentiation, peptides preventing cell differentiation, preferably stem cell differentiation, peptides promoting cell adhesion, peptides promoting cell survival, peptides promoting cell proliferation, peptides promoting cell expansion, peptides supporting migration and/or motility, and peptides promoting cell cycle progression,

wherein the, one, two, three or more or all polypeptide(s) b) is/are conjugated to the polymer a), wherein the conjugation is realized by a linkage at the one or more functional groups(s) of polymer a).
2. Cell attachment polymer conjugate according to claim 1, wherein the one or more polypeptide(s) exhibit a sequence identity of at least 70 %, preferably at least 80 %, preferably at least 90 %, preferably at least 91 %, preferably at least 92 %, preferably at least 93 %, preferably at least 94 %, preferably at least 95 %, preferably at least 96 %, preferably at least 97 %, preferably at least 98 %, preferably at least 99 % to a sequence of equal length of an integrin binding protein, preferably of collagen, laminin, tenascin, vitronectin or fibronectin.

3. Cell attachment polymer conjugate according to claim 1 or 2, wherein the one or more polypeptide(s) have an amount of amino acids is in a range of from 5 to 95 amino acids, preferably in the range of from 5 to 90 amino acids, preferably in the range of from 5 to 85 amino acids, preferably in the range of from 5 to 80 amino acids, preferably in the range of from 5 to 75 amino acids, preferably in the range of from 5 to 70 amino acids, preferably in the range of from 6 to 65 amino acids, preferably in the range of from 6 to 60 amino acids, preferably in the range of from 6 to 55 amino acids, preferably in the range of from 6 to 50 amino acids, preferably in the range of from 6 to 45 amino acids, preferably in the range of from 6 to 40 amino acids, preferably in the range of from 7 to 35 amino acids, preferably in the range of from 7 to 30 amino acids.
4. Cell attachment polymer conjugate according to any one of the previous claims, wherein the ULA polymer a) is selected from the group consisting of block-copolymers, brush polymers, such as polyethylene glycol-polypropylene oxide or ethylene glycol-propylene oxide, n-Butylmethacrylat with methacrylic acid or alkyl-containing brush polymers, such as copolymers of Poly-(ethylenglykol)-methylether-methacrylat and propargyl acrylate, alternating styrene maleic anhydride copolymers such as esterified block- copolymers.
5. Cell attachment polymer conjugate according to any one of the previous claims, wherein the cell attachment polymer conjugate comprises covalent bonds between the ultra-low attachment (ULA) polymer (a) and the one or more polypeptide(s) (b).
6. Cell attachment polymer conjugate according to any one of the previous claims, wherein the one or more polypeptide(s) is/are or correspond to fragments of proteins of the human extracellular matrix (ECM).
7. Method for manufacturing a cell attachment polymer conjugate according to any one of claim 1 to 6 comprising the steps of:
  - i) providing at least one ULA polymer carrying one or more functional groups(s), wherein the, one, two, three or more or all functional groups(s) is/are selected from the group consisting of primary amines, carboxyl groups, aldehyde groups, and epoxide groups, hydroxyl groups, ketones, esters, amides, nitriles, thiols, hydroxylamines, imines, alkenes, isocyanates, halides, ether and azides,

- ii) providing one or more polypeptide(s), wherein the amount of amino acids in the polypeptide(s) is in a range of from 5 to 100 amino acids,

wherein the, one or more polypeptide(s) is/are selected from the group consisting of growth peptides, peptides stimulating cell differentiation, preferably stem cell differentiation, peptides preventing cell differentiation, preferably stem cell differentiation, peptides promoting cell adhesion, peptides promoting cell survival, peptides promoting cell proliferation, peptides promoting cell expansion, peptides supporting migration and/or motility, and peptides promoting cell cycle progression,

- iii) conjugating the, one or more polypeptide(s) provided in step ii) to the at least one ULA polymer provided in step i).

- 8. Device for cultivating cells, wherein the device comprises a surface, which is coated with a cell attachment polymer according to any one of claim 1 to 6.
- 9. Device for culturing cells according to claim 8, wherein the device is selected from the group consisting of flasks, such as round bottom flasks, spinner flasks, Erlenmeyer flasks, retorts and Florence flasks, cell culture dishes, cell culture bottles, cell culture bags, pipette tips, petri dishes, multiwell plates, reactors, especially bioreactors, tubes, pipettes, syringes, chips, slides, and cell culture scaffolds, such as microcarriers.
- 10. Device for culturing cells according to claim 8 or 9, wherein the device is made of a material selected from the group consisting of glass, quartz, silicon, metals, metal oxides, and organic polymers, such as polycarbonate, polystyrene, hydrophilized polystyrene, polyamide, poly(methyl methacrylate), polyesters, polyvinylchloride, polyvinylidene chloride, polymers comprising fluorinated monomers like fluorinated ethylene or propylene, polyolefines, such as polyethylene, polypropylene, polynorbornene, cyclic olefin polymers or cyclic olefin copolymers such as copolymers of ethylene and norbornene, or mixtures thereof.
- 11. Device for culturing cells according to any one of claims 8 to 10, wherein the device is made of a material selected from the group consisting of polyolefines, in particular polystyrene, polyethylene, polypropylene, and (partially) fluorinated polyolefines such as fluorinated ethylene propylene.

12. Method for manufacturing of a cell culture device, preferably a cell culture device according to any one of claims 8 to 10, comprising the steps of:
  - a) providing a device for cultivating cells,
  - b) providing a polymer conjugate according to any one of claims 1 to 6, and
  - c) coating the device, preferably the surface of the device, provided in step a) with the polymer conjugate provided in step b).
13. Method according to claim 12, wherein the wherein the device is made of a material selected from the group consisting of glass, quartz, silicon, metals, metal oxides, and organic polymers, such as polycarbonate, polystyrene, hydrophilized polystyrene, polyamide, poly(methyl methacrylate, polyesters, polyvinylchloride, polyvinylidene chloride, polymers comprising fluorinated monomers like fluorinated ethylene or propylene, polyolefines, such as polyethylene, polypropylene, polynorbornene, cyclic olefin polymers or cyclic olefin copolymers such as copolymers of ethylene and norbornene, or mixtures thereof.